

WHAT IS CLAIMED IS:

1. A method of determining a nucleotide sequence of an analytical oligo nucleic acid for use in analysis of the nucleic acid, comprising the steps of:

5 listing all unit nucleotide sequences present on a target nucleic acid to be analyzed and having a predetermined length which is shorter than the analytical oligo nucleic acid to be designed; and

10 extracting a nucleotide sequence containing a sequence occurring at a low frequency on the target nucleic acid from the candidate sequences of the analytical oligo nucleic acids, as an analytical sequence suitable for analysis for the nucleotide sequence of the target nucleic acid, on the basis of  
15 occurrence frequency of the individual unit sequences listed.

2. The method according to claim 1, wherein the extraction step is performed by successively applying a plurality of different processes.

20 3. The method according to claim 1, wherein the extraction step further comprises a step of selecting the candidate sequences on the basis of stability of a molecular structure of the each oligo nucleic acids formed of the candidate sequences.

25 4. The method according to claim 3, wherein the stability of a molecular structure is thermal stability.

5. The method according to claim 3, wherein the stability of a molecular structure is a melting temperature ( $T_m$ ) of the candidate sequences and/or stability of an intramolecular secondary structure formed of the candidate sequences.

6. A method of determining a nucleotide sequence of an analytical oligo nucleic acid for use in analysis of a nucleic acid, comprising:

a first calculation step of calculating a occurrence frequency of each of  $n$  unit sequences occurring on a nucleotide sequence of a target nucleic acid to be analyzed on the basis of a value of  $4^n$  which correspond to all of the  $n$  unit sequences formed of  $n$  nucleotide sequences ( $n$  is an integer of 2 or more);

a first extraction step of extracting a sequence having  $p$  number of nucleotides and present on the nucleotide sequence of a target nucleic acid, said  $p$  is larger than  $n$  by  $m$  ( $m$  is an integer of 1 or more);

a second calculation step of extracting  $n$  unit sequences occurring on the candidate sequence extracted in the first extraction step and obtaining a occurrence frequency index of the candidate sequence on the nucleotide sequence of the target nucleic acid on the basis of the occurrence frequency of each of the  $n$  unit sequences obtained in the first calculation step; and

a second extraction step of selecting a single or a plurality of candidate sequences having a low

occurrence frequency index obtained in the second calculation step as potential candidate sequences.

7. The method according to claim 6, wherein said n is 5, 6, or 7.

5           8. The method according to claim 6, further comprising a third extraction step of selecting a candidate sequence having a low stability on the basis of stability of a molecular structure of each of oligo nucleic acid molecules formed of the potential  
10 candidate sequences.

9. The method according to claim 8, wherein the stability of a molecular structure is a magnitude of  $T_m$  value and/or stability of an intramolecular secondary structure.

15           10. The method according to claim 9, wherein, in said third extraction step, a sequence having the  $T_m$  value falling within a predetermined range is selected from the potential candidate sequences and forming an unstable secondary structure is further selected.

20           11. The method according to any one of claims 1 to 10, wherein all necessary steps are sequentially performed by a computer.

12. The method according to any one of claims 1 to 10, wherein said analytical sequence is a nucleotide  
25 sequence of an analytical oligo nucleic acid used in a method for detecting a specific nucleotide sequence present in a nucleotide sequence of a nucleic acid by

using an enzyme reaction which requires hybridization reactions of nucleic acid, or used in a hybridization reaction of the nucleic acid.